Development of a Site-Specific Criterion For Copper Downstream of the Butler County Queens Acres Wastewater Treatment Plant

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prepared by:

Great Lakes Environmental Center

Great Lakes Environmental Center
1295 King Avenue
Columbus, Ohio 43212

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DEVELOPMENT OF A SITE-SPECIFIC CRITERION FOR COPPER DOWNSTREAM OF THE BUTLER COUNTY QUEENS ACRES WASTEWATER TREATMENT FACILITY USING A WATER-EFFECT RATIO

I. INTRODUCTION

A water-effect ratio (WER) determination was conducted for the modification of the State of Ohio's water quality chronic criterion for copper in the Indian Creek downstream of the Butler County Queens Acres Wastewater Treatment Plant (QAWWTP) effluent discharge in Hamilton, Ohio. This report presents the methods and results of three WER determinations conducted on surface water and effluent samples collected from QAWWTP's final effluent and the Indian Creek on May 10-11, July 5-6 and August 28-29, 2011. For all three sampling events, WERs were determined using side-by-side acute toxicity tests conducted with the primary test species, *Ceriodaphnia dubia* (*C. dubia*), exposed to copper in two different dilution waters; site water (simulated downstream receiving water) and laboratory water (moderately hard reconstituted water). For the July 5-6, 2011 sampling event, a WER was also determined using the secondary test species, *Pimephales promelas* (fathead minnow) 48-hour acute toxicity test. The toxicity tests conducted for the WER determinations were performed at Great Lakes Environmental Center's (GLEC) laboratory in Columbus, Ohio.

II. METHODS

A. Site Water

Simulated downstream water was used as the site water for the WER determinations. Simulated downstream water consisted of a mixture of final effluent and upstream receiving water (Indian Creek), with the proportions characterized as a Type 1 or Type II WER according to the seasonal downstream conditions prevalent during the sampling. The Type I WERs, where the downstream condition is between 1 and 2 times the design flow, were conducted in July and August. The Type II WER, which is for downstream conditions between 2 and 10 times the design flow, occurred in May. The percentage of effluent and upstream Indian Creek water that were used to prepare the simulated downstream site water for the three WER determinations were as follows:

Sample Date	Type I or II WER	Percent of effluent	Percent of Upstream Indian Creek Water
May 10-11, 2011	Type II	10	90
July 5-6, 2011	Type I	50	50
August 28-29, 2011	Type I	100	0

The objective of performing the WER in August without using upstream Indian creek samples was to assess the WER in a simulated worst case receiving water situation where low flow conditions would result in the downstream water consisting of 100 percent effluent with no dilution from the upstream water.

For the three collection dates, 24-hour composite samples of the final effluent and grab samples of upstream Indian creek water were collected by QAWWTP staff in polyethylene cubitainers and placed in coolers containing wet ice. The samples were delivered by QAWWTP staff to GLEC's Columbus, Ohio laboratory. Upon receipt, the samples were logged in, given a GLEC identification number, and measured for temperature, pH, dissolved oxygen, specific conductivity, hardness and alkalinity. Copies of the chain-of-custody forms for the three sampling events and the effluent characterization forms containing the initial water quality measurements are presented in Appendix A.

Summaries of the water quality characteristics of the QAWWTP effluent, upstream Indian creek water and the simulated downstream water mixture for the three sampling events are given in Tables 1, 2 and 3 respectively.

B. Laboratory Water

Diluted Mineral Water (DMW) was used as dilution water for the *C. dubia* laboratory water tests. DMW was prepared using GLEC's Standard Operating Procedure (SOP), which is based on instructions given in EPA (2002). The base water used to prepare the reconstituted water was deionized tap water. Perrier brand sparkling mineral water was added in the appropriate amount to deionized water and mixed and aerated at room temperature hours prior to use. The DMW batch numbers used in the May, July and

September WER determinations were 1583, 1589 and 1598 respectively (copies of the DMW preparation forms are given in Appendix B).

A summary of the water quality characteristics for the three DMW batches is given in Table 4.

Moderately Hard Reconstituted Water (MHW) was used as dilution water for the laboratory water test with fathead minnows. MHW was prepared using GLEC's Standard Operating Procedure (SOP), which is based on instructions given in EPA (2002). The base water used to prepare the reconstituted water was deionized tap water. Reagent grade salts were added in the appropriate amounts to deionized water and mixed at room temperature. The MHW batch number used in the July WER determination was 2241 (copies of the reconstituted water preparation form is given in Appendix B).

A summary of the water quality characteristics for the MHW batch is given in Table 4.

C. Stock and Test Solution Preparation

Reagent grade cupric sulfate, five hydrate (Fisher Chemicals, certified A.C.S.; Lot No. 045279) was used to make all stock and test solutions in all WER determinations. The day the WER tests were initiated, a stock solution was prepared by dissolving cupric sulfate in deionized water. Test solutions (the solutions to which the test organisms were exposed) were prepared the day the WER tests were initiated according to the following sequences:

Laboratory Water

- The spiked test solutions were serially diluted using a 0.7X dilution factor.
- The spiked test solutions were allowed to equilibrate for 3 hours.

Site Water

- An appropriate volume of stock solution was added to a measured volume of the effluent and mixed using a 0.7X dilution factor.
- The spiked effluent test solutions were allowed to equilibrate for 2 hours.
- After two hours, upstream Indian creek water was added to each effluent dilution so as to obtain the mixture proportion based upon the predetermined site water concentrations.

- The effluent and receiving water were mixed and allowed to equilibrate for approximately 2 hours before initiating the tests.

A detailed description of the stock and test solution preparation procedures for the three WER determinations is given in the laboratory information sheets in Appendix C.

D. Toxicity Test Procedures

1. Primary Toxicity Test

The primary toxicity test, the *C. dubia* 48-hour static acute toxicity test, was used to determine a WER for the May, July and August sampling events. All procedures followed the study-specific SOP for conducting a 48-hr *C. dubia* static acute toxicity test, as presented in the Study Plan for this project. A summary of the test conditions is as follows:

test chamber: 50 ml plastic cups
depth of test solution: 32 mm
volume of test solution: 30 ml
number of organisms/chamber: 5
lighting: 16-hour light/8-hour dark photoperiod; 10-20 $\mu E/m^2/s$

test initiation:	May WER determination, May 12, 2011 at 1400 hours July (WER determination, July 7, 2011 at 1450 hours August WER determination, August 30, 2011 at 1415 hours
test termination:	May WER determination, May 14, 2011 at 1600 hours July WER determination, July 9, 2011 at 1445 hours August WER determination, September 1, 2011 at 1430 hours

2. Secondary Toxicity Test

The secondary toxicity test, the fathead minnow 48-hour static acute toxicity test, was used to determine a WER for the July sampling event. All procedures followed the study-specific SOP for conducting a fathead minnow 48-hour static acute toxicity test, as presented in the Study Plan for this project. A summary of the test conditions is as follows:

test chamber: 500 ml glass beaker

depth of test solution: 38 mm

volume of test solution: 250 ml

number of organisms/chamber: 10

lighting: 16-hour light/8-hour dark photoperiod; 10-20 $\mu E/m^2/s$

test initiation: July (fathead minnow) WER determination, July 7, 2011 at 1530 hours

test termination: July (fathead minnow) WER determination, July 9, 2011 at 1545 hours

3. Test Organisms

Ceriodaphnia dubia

Stock cultures of *C. dubia* used in the WER study were originally obtained from Aquatic BioSystems located in Fort Collins, Colorado. The *C. dubia* used in the WER study were cultured at GLEC in 30 ml vessels containing 1 adult and 20 ml of natural source water and maintained in environmental chambers under controlled conditions (temperature 25 ± 1°C; photoperiod 16 h light and 8 h dark). Three times a week, cultures were transferred to fresh water containing 2.0 ml of a yeast/trout food/Cerophyl® (YTC) food suspension (see EPA-821-R-02-012 for procedures for preparing the food suspension), and 2.5 ml of 2.3×10^8 cells/ml of the green alga, *Pseudokirchneriella subcapitata*, per liter. The days the culture water was not changed, each vessel received 1 drop each of the YTC suspension and algae. Survival and reproduction of culture animals were monitored and general water chemistry measurements (dissolved oxygen, pH, temperature and specific conductivity) were made and recorded each time the culture water was changed. Test animals were obtained from cultures where survival of culture animals exceeded 80 percent, and which produced at least three broods per female. Twenty-four hours prior to test initiation, all young were removed from the culture chambers to ensure that only daphnids less than 24-h old would be available to initiate the test. Reference toxicant information for *C. dubia* is provided in Appendix D.

Fathead Minnows

Two day old fathead minnows were used for the WER determination conducted July 7-9, 2011 were obtained from Aquatic BioSystems located in Fort Collins, Colorado and were cultured at temperature, 25 ± 1°C; photoperiod and 16-hours light: 8-hours dark; light intensity, 10-20 $\mu E/m^2/s$. Fathead minnows used for testing were received on July 6, 2011 (one day prior to testing) at 23.2°C and were place in a one gallon plastic bucket and fed live *Artemia nauplii* (brine shrimp). At the end of the day, the fish were transferred into fresh moderately hard water and placed inside an environmental chamber to acclimate to laboratory test conditions. On the day of test initiation, the fathead minnows were again fed live brine shrimp and then transferred to fresh moderately hard water prior to testing.

4. Measurements

A number of water quality parameters, as well as total recoverable and dissolved copper, were measured in the effluent and receiving water samples and in the test solutions of the 48-hour WER toxicity tests. The types of chemical measurements made in the surface water samples and in the toxicity test solutions, and the methods used to perform the measurements are presented in Table 5. Copies of the data sheets containing the analytical results of the water quality parameters conducted by Belmont Labs are provided in Appendix E.

III. RESULTS

A. Primary Toxicity Tests

The average and range of pH, dissolved oxygen, temperature and specific conductivity in the test solutions of the primary toxicity tests conducted May 12-14, July 7-9 and August 30 - September 1, 2011 are given in Tables 6, 7 and 8 respectively. The copper and *C. dubia* 48-hour survival measurements for the laboratory water and site water toxicity tests for the WER determinations are given in Tables 9 through 14. The measured dissolved and total copper concentrations were very similar to the anticipated nominal copper concentrations in the three DMW tests. Dissolved and total copper concentrations in the site water tests were typically higher than the nominal due to the presence of background copper in the site waters. The dissolved copper concentrations measured at test initiation (Day 0) and at test termination (Day 2) did not differ appreciably, and were similar to the total recoverable copper concentrations in all the tests.

The Probit and Spearman-Karber methods were used to calculate LC_{50} values using the nominal, total recoverable and dissolved copper concentrations. The geometric mean of the dissolved copper concentrations measured at Day 0 and Day 2 were the dissolved copper concentrations used in the LC_{50} calculations. For the May 12-14, 2011 WER determination, the LC_{50} values for the DMW and the site water tests, based on dissolved copper concentrations, were 6.257 and 38.04 µg/L, respectively (Tables 9 and 10). For the July 7-9, 2011 WER determination, the LC_{50} values based on dissolved copper concentrations for the DMW and site water tests were and 6.710 and 123.1 µg/L, respectively (Tables 11 and 12). For third WER determination conducted August 30 - September 1, 2011, the LC_{50} values for the DMW and site water tests, based dissolved copper concentrations, were 4.178 and >218.5 µg/L, respectively (Tables 13 and 14). All DMW and site water data sets showed a typical concentration-response relationship

The LC_{50} values for the DMW tests (6.257, 6.710 and 4.178 µg/L) are similar to the dissolved copper LC_{50} values for *C. dubia* in laboratory waters of similar hardness. The average and range of the seven LC_{50} values listed in the streamlined WER guidance (EPA-822-R-01-005) that were conducted in laboratory waters with hardness values similar to DMW (80 to 90 mg/L as $CaCO_3$) was 12.75 µg/L and 6.98 to 25.25 µg/L, respectively. All site water and laboratory water data sets showed a typical concentration-response relationship, and all tests had acceptable control survivorship (≥95 percent).

Copies of the data sheets containing the individual physical, chemical and survival measurements and printouts of the statistical analyses are given in Appendix F.

B. Secondary Toxicity Test

The average and range of pH, dissolved oxygen, temperature and specific conductivity in the test solutions of the secondary toxicity tests conducted July 7-9, 2011 are given in Table 15. The copper and fathead minnow 48-hour survival measurements for the MHW and site water toxicity tests for the WER determination are given in Tables 16 and 17. The total recoverable copper measurements were near the target nominal concentrations for both tests, while the dissolved measurements for the MHW were also close to nominal concentrations. The site water dissolved copper concentrations measurements were not appreciably different from the nominal concentrations with the exception of the two highest measured concentrations that were approximately ≥43 percent lower than the nominal concentrations. The difference between the nominal and measured dissolved values of the two highest test concentrations is attributed to reduced copper solubility at higher concentrations. The LC_{50} values for the Moderately MHW and site water tests, based on dissolved copper concentrations, were 88.10 and >1220 µg/L, respectively (Tables 16 and 17).

Copies of the data sheets containing the individual physical, chemical and survival measurements and printouts of the statistical analyses are given in Appendix F.

C. Calculation of WER

Tables 18 and 19 present water hardness values, as well as laboratory water, site water, and the adjusted laboratory water LC_{50} values used to calculate the WERs based on dissolved and total recoverable copper respectively. For calculation of the adjusted laboratory water LC_{50}, the hardness slope of 0.9422 was used to adjust the LC_{50} of the tests performed in laboratory water to the hardness of the site water. The adjusted WERs were calculated by dividing the site water LC_{50} by the adjusted laboratory water LC_{50}. An example of the calculation that was used in the determination of the WERs is provided below:

From Table 18 (dissolved copper), the May *C. dubia* WER was based upon:
Lab water hardness = 84 mg/L
Site water hardness = 269.3 mg/L
Lab water LC_{50} = 6.257 µg/L
Site water LC_{50} = 38.04 µg/L

Adjustment of the LC_{50} to lab water hardness of 84 mg/L and a site water hardness of 269.3 mg/L:

$$\text{Hardness adjusted lab water } LC_{50} = 6.257 \times (269.3 \div 84)^{0.9422} = 18.75$$

WER = site water LC_{50} (38.04) ÷ hardness adjusted lab water LC_{50} (18.75) = 2.028

D. Calculation of hWER

Summaries of the measurements used to determine the highest WERs (hWERs) for dissolved and total recoverable copper are presented in Tables 20 and 21. Definitions of the measurements and calculations that were used in the determination of the hWERs are as follows:

eFLOW: the flow of the effluent that was the basis of the preparation of the simulated downstream water.

uFLOW: the flow of the upstream water that was the basis of the preparation of the simulated downstream water.

uCONC: the concentration of copper in the sample of upstream water used in the preparation of the simulated downstream water.

HCME: Highest concentration of the copper that could be in the effluent without causing the concentration of the metal downstream to exceed the site-specific criterion. The HCME is used in the calculation of the hWER and is determined using the following equation:

$$\mathrm{HCME} = \frac{\{\,[(\mathrm{CCC})(\mathrm{WER})(\mathrm{eFLOW} + \mathrm{uFLOW})] - [(\mathrm{uCONC})(\mathrm{uFLOW})]\,\}}{\{\,\mathrm{eFLOW}\,\}}$$

where, CCC = the Ohio EPA CCC (OMZA) for dissolved and total recoverable copper to be adjusted using the hardness value of the site water.

hWER: Highest Water-Effect Ratio. Used in the determination of the FWER, and calculated using the following equation:

$$\mathrm{hWER} = \frac{\{\,(\mathrm{HCME})\,(\mathrm{eFLOWdf}) + (\mathrm{uCONCdf})\,(\mathrm{uFLOWdf})\,\}}{\{\,(\mathrm{CCC})\,(\mathrm{eFLOWdf} + \mathrm{uFLOWdf})\,\}}$$

where, df = design flow, and the hardness concentration used for the Ohio EPA CCC equation was 264 mg/L as $CaCO_3$ (hardness at the design flow condition).

An example of the calculations that were used in the determination of the hWERs is provided below:

From Table 20 (dissolved copper), the May *C. dubia* hWER was based upon:

Dissolved CCC (OMZA) = 20.5 μg/L

eFlow = 0.1 cfs

uFlow = 0.9 cfs

uConc = 1.26 μg/L (upstream copper concentration of May sample)

eFlowdf = 0.928 cfs

uConcdf = 1.1 μg/L (mean of the upstream copper concentrations for samples collected)

uFlowdf = 0.1 cfs

HCME = [(20.5)(2.028)(0.1 + 0.9)] - [(1.26)(0.9)] ÷ [0.1] = 404.4 μg/L

hWER = [(404.4)(0.928) + (1.1)(0.1)] ÷ [(20.5)(0.928 + 0.1)] = 17.81

Based upon the data presented above for the primary test species, the dissolved copper hWERs for the May, July and August samples were 17.81, 13.04 and 24.76, respectively

IV. RECOMMENDATION AND DISCUSSION

Based on the "Interim Guidance on Determination and Use of Water-Effect Ratios for Metals" (EPA 1994), the Final WER (FWER) is determined as follows:

> "the FWER should be derived from the WERs and hWERs using the lowest numbered option whose requirements are satisfied:
>
> 1. If there are two or more Type I WERs:
>   a. If at least 19 percent of all of the WERs are the properties of the Type WERs:
>   1) If the range of the Type I WERs is not greater than a factor of 5 and/or the range of the ratios of the Type I WER to the concentration of metal in the simulated downstream water is not greater than a factor of 5, the FWER is the lower of (a) the adjusted geometric mean of all of the Type I WERs and (b) the lowest hWER."

A. Final Determination of the FWER for Dissolved Copper

1. The adjusted geometric mean of all of the Type I dissolved copper WERs (July and August samples) is 8.684
2. The lowest hWER (excluding the secondary species hWER) in Table 20 is 13.04

Based on the language provided above, the FWER for dissolved copper is 8.684

B. Final Determination of the FWER for Total Recoverable Copper

1. The adjusted geometric mean of all of the Type I total recoverable copper WERs (July and August samples) is 9.302
2. The lowest hWER (excluding the secondary species hWER) in Table 21 is 14.24

Based on the language provided above, the FWER for total recoverable copper is 9.302

Based on the results obtained in this study, GLEC recommends the FWERs for dissolved and total copper of 8.684 and 9.302, respectively.

C. Assessment of the FWER's for Modification of the QAWWTP NPDES Permit

Based on the results obtained in this study, GLEC recommends the FWERs for dissolved and total copper of 8.684 and 9.302, respectively. Butler County, in accordance with its NPDES permit for the QAWWTP, can now initiate a permit modification request to the Ohio EPA for total copper using the calculated FWER value of 9.302. Specifically, this FWER can be applied by multiplying the applicable copper Aquatic Life Criteria (monthly average of 21.4 μg/L and daily maximum of 34.8 μg/L) for the surface water into which QAWWTP discharges, Indian Creek. Applying the FWER for total copper results in a site-specific Aquatic Life Criteria monthly average of 199.1 μg/L and a daily maximum of 323.7 μg/L, which remain the most restrictive applicable water quality standard(s). Given QAWWTP's projected effluent quality (PEQ) values for total copper (24.8 μg/L average, and 48.3 μg/L maximum), total copper would be considered a "group 2" parameter by Ohio EPA (<25% of the site-specific criterion). Discharge limitations are not recommended for Group 2 parameters, and monitoring is optional.

V. BIOTIC LIGAND MODEL (BLM) RESULTS

In water, chemical constituents can mitigate copper toxicity by either competing with copper for binding sites at the route of exposure for aquatic organisms or by forming metal complexes with copper and rendering it biologically unavailable. As a function of the chemical constituent in water that either compete with or bind to copper, the Biotic Ligand Model (BLM) can be used to calculate copper toxicity to aquatic organisms. Calculation of the BLM requires the input of nine chemical parameters as well as temperature (Carlton 2008).

Results of the BLM for the three laboratory and three site water samples to estimate dissolved copper LC_{50} values, are given in the text table below. The LC_{50} values estimated by the BLM (HydroQual 2011) were generally higher for the laboratory water tests and were comparable for the site water tests. An important input parameter of the BLM is dissolved organic carbon (DOC). DOC for laboratory water is typically below the detection level of 1 mg/L as was the case for the May and July samples. The August sample had a measurement of 1.73 mg/L which was considered anomalous based on a comparison to previous laboratory water samples. If ¼ of the detection level is used rather than ½ the detection level, the predicted LC_{50} values are very close to the actual laboratory water LC_{50} values. The site water LC_{50} values predicted by the BLM were similar to the actual LC_{50} values for the two *C. dubia* tests (May and July) that did not have a greater than value (>). The BLM estimates for these two samples were within 1.2X of the actual LC_{50} values (see text table below). For the two tests that had > values (*C. dubia* site water August sample, fathead minnow site water July sample), mortality was observed in the highest test concentration suggesting the LC_{50} would be a little higher than the values given in this report. Assuming the actual LC_{50} values were not meaningfully higher for the two > values, the BLM estimates would be within 2X of the actual tested LC_{50} values. The BLM therefore appears to provide a reasonable estimate of the site water LC_{50} values. The input parameters to the BLM, as well as the projected and actual LC_{50} values are provided in the table below.

Biotic Ligand Model Results[a]

BLM Parameter	Laboratory water sample				Site water sample			
	Ceriodaphnia dubia			FHM[b]	*Ceriodaphnia dubia*			FHM[b]
	May	**July**	**August**	**July**	**May**	**July**	**August**	**July**
Ca, mg/L	33.6	33.6	30.5	15.0	74.5	60.9	42.6	42.6
Mg, mg/L	1.19	1.26	1.20	12.9	20.9	21.1	14.4	14.4
K, mg/L	<0.100	0.105	<0.0057	1.8	1.78	6.65	16.3	16.3
Na, mg/L	2.28	2.15	1.84	23.8	15.5	63.3	150	150
Cl, mg/L	4.79	4.74	5.02	<4.00	23.2	63.9	111	111
DOC, mg/L	<1.00	<1.00	1.73	<1.00	1.28	3.79	5.07	5.07
SO_4, mg/L	7.32	8.02	7.72	98.0	20.2	33.2	45.6	45.6
Sulfide, mg/L	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00
Alkalinity, mg/L as $CaCO_3$	68.00	68.00	68.00	60.0	220	180	116	116
Temp, °C	25.10	24.40	24.30	25.0	24.7	24.5	24.8	24.9
pH, S.U.	7.90	7.80	7.40	7.7	8.3	8.1	7.7	8.1
BLM LC_{50}, dissolved µg/L Cu	15.55	15.09	11.64	132.3	44.38	118.9	130.6	1173
Test LC_{50}, dissolved µg/L Cu	6.257	6.71	4.178	88.10	38.04	123.1	>218.5	>1220

[a] For < values, ½ the detection level was used in the BLM. For sulfide, 0.001 mg/L was used. The 1.73 mg/L measurement of DOC in the August laboratory water was considered anomalous and 0.5 mg/L was used instead for the BLM calculation.
[b] FHM – fathead minnow

The Interim WER Guidance (USEPA 1994) recommends periodic reevaluation of the WER. For WERs determined using downstream water, as is the case for the Butler County WER, effluent is more influential than upstream water. Downstream WERs should be reevaluated whenever newly implemented controls or other changes might substantially impact the effluent, i.e., might impact the forms and concentrations of the chemical, hardness, alkalinity, pH, suspended solids, organic carbon, or other toxic materials. A special concern is the possibility of a shift from the discharge of a nontoxic form of the chemical to the discharge of a toxic form of the chemical, such that the concentration of the chemical does not increase.

For periodic reevaluations of the Butler County WER, a BLM estimate of the *C. dubia* LC_{50} would provide a cost-effective approach for determining if the effluent would produce a WER comparable to that determined in this study.

VI. REFERENCES

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TABLE 1. WATER QUALITY CHARACTERISTICS OF THE EFFLUENT USED IN WATER EFFECT RATIO DETERMINATIONS.

Chemical Parameter	**May Effluent**	**July Effluent**	**August[A] Effluent**
Hardness (mg/L $CaCO_3$)	260.0	216.0	169.3
Alkalinity (mg/L $CaCO_3$)	224.0	168.0	116.0
pH (SU)*	NA	NA	7.7
Dissolved Organic Carbon (mg/L)	3.06	4.21	5.07
Total Suspended Solids (mg/L)	<5.00	<5.00	<2.47
Total Recoverable Copper (µg/L)	12.0	30.3	36.1
Dissolved Copper (µg/L)	11.7	31.3	35.4

* pH was measured at test temperature (25±1°C)

NA Not Available

A The August effluent chemistries are equivalent to the August site water chemistries found in table 3.

TABLE 2. WATER QUALITY CHARACTERISTICS OF THE UPSTREAM USED IN WATER EFFECT RATIO DETERMINATIONS.WATER QUALITY CHARACTERISTICS OF HE EFFLUENT USED IN WATER EFFECT RATIO DETERMINATIONS.

Chemical Parameter	**May Upstream**	**July Upstream**
Hardness (mg/L $CaCO_3$)	256.0	232.0
Alkalinity (mg/L $CaCO_3$)	208.0	192.0
pH (SU)*	NA	NA
Dissolved Organic Carbon (mg/L)	1.37	1.46
Total Suspended Solids (mg/L)	<5.00	6.00
Total Recoverable Copper (µg/L)	1.51	0.91
Dissolved Copper (µg/L)	1.26	0.93

TABLE 3. WATER QUALITY CHARACTERISTICS OF THE SITE WATER USED IN WATER EFFECT RATIO DETERMINATIONS.

Chemical Parameter	**May Site Water**	**July Site Water**	**August Site Water**
Hardness (mg/L $CaCO_3$)	269.3	225.3	169.3
Alkalinity (mg/L $CaCO_3$)	220.0	180.0	116.0
pH (SU)*	8.3	8.1	7.9
Dissolved Organic Carbon (mg/L)	1.28	3.79	5.07
Total Suspended Solids (mg/L)	<5.00	6.00	<2.47
Total Recoverable Copper (µg/L)	2.46	15.5	36.1
Dissolved Copper (µg/L)	2.51	15.2	35.4

* pH was measured at test temperature (25±1°C)

TABLE 4. WATER QUALITY CHARACTERISTICS OF DMW AND MODERATELY HARD LABORATORY WATER USED IN WATER-EFFECT RATIO DETERMINATIONS.

Chemical Parameter	**May DMW**	**July DMW**	**July Mod. Hard**	**August DMW**
Hardness (mg/L $CaCO_3$)	84.0	84.0	84.0	85.3
Alkalinity (mg/L $CaCO_3$)	68.0	68.0	60.0	68.0
pH (SU)*	7.9	7.8	7.7	7.7
Dissolved Organic Carbon (mg/L)	<1.0	<1.0	<1.0	1.73
Total Suspended Solids (mg/L)	<5.00	<5.00	<5.00	<2.47
Total Recoverable Copper (µg/L)	<0.406	<0.406	<0.406	<0.406
Dissolved Copper (µg/L)	<0.406	<0.406	<0.406	<0.406

* pH was measured at test temperature (25±1°C)

TABLE 5. SAMPLING SCHEDULE AND METHOD FOR CHEMICAL MEASUREMENTS.

Parameter	Sample Type(s)	Sampling Schedule	Method
pH	Diluted Mineral Water Site Waters	Sample Receipt	APHA – 4500 H+
	WER Test	Test initiation and test termination	
Specific Conductivity	Diluted Mineral Water Site Waters	Sample Receipt	APHA - 2510
	WER tests	Test initiation and test termination	
Dissolved Oxygen (DO)	Diluted Mineral Water Site Waters	Sample Receipt	APHA – 4500-OG
	WER tests	Test initiation and test termination	
Total Hardness	Diluted Mineral Water Site Waters	Sample Receipt	EPA 130.2
Total Alkalinity	Diluted Mineral Water Site Waters	Sample Receipt	APHA - 2320
Calcium, Magnesium, Sodium, Potassium	Diluted Mineral Water Site Waters	Sample Receipt	EPA 200.7
Sulfate, chloride	Diluted Mineral Water Site Waters	Sample Receipt	EPA 375.4, SM 4500-CL-E
Sulfide	Diluted Mineral Water Site Waters	Sample Receipt	EPA 376.1/ SM 4500S2-F
Total Recoverable Copper	Diluted Mineral Water Site Waters	Sample Receipt	EPA 200.7
	WER tests	Test initiation	
Dissolved Copper	Diluted Mineral Water Site Waters	Sample Receipt	EPA 200.7 (after 0.45 µm filtration)
	WER tests	Test initiation Test Termination	
Total Suspended Solids (TSS)	Diluted Mineral Water Site Waters	Sample Receipt	EPA 160.2/SM 2540
Dissolved Organic Carbon (DOC)	Diluted Mineral Water Site Waters	Sample Receipt	SM 5310C (filtered)

TABLE 6. AVERAGE AND RANGE OF WATER QUALITY CHEMICAL MEASUREMENTS IN TEST SOLUTIONS OF WATER-EFFECT RATIO ACUTE TOXICITY TESTS CONDUCTED ON MAY 12-14, 2011 USING *Ceriodaphnia dubia*.

PARAMETER	Laboratory Water (DMW)		Site Water (10% effluent+90%upstream)	
	Average	Range	Average	Range
pH, SU	7.9	7.9 - 8.0	8.3	8.1 - 8.4
Dissolved Oxygen, mg/L	7.6	7.4 - 7.7	7.9	7.5 – 8.5
Temperature, °C	25.1	25.0 - 25.4	24.7	24.2 - 25.4
Specific Conductivity, μmhos/cm	188	183 - 192	547	515 - 574

TABLE 7. AVERAGE AND RANGE OF WATER QUALITY CHEMICAL MEASUREMENTS IN TEST SOLUTIONS OF WATER-EFFECT RATIO ACUTE TOXICITY TESTS CONDUCTED ON JULY 7-9, 2011 USING *Ceriodaphnia dubia*.

PARAMETER	Laboratory Water (DMW)		Site Water (50% effluent+50%upstream)	
	Average	Range	Average	Range
pH, SU	7.8	7.7 – 7.9	8.1	7.9 - 8.5
Dissolved Oxygen, mg/L	7.6	7.3 - 7.8	7.3	6.6 - 8.3
Temperature, °C	24.4	24.0 - 24.9	24.5	24.0 - 24.9
Specific Conductivity, μmhos/cm	182	171 - 189	732	685 - 760

TABLE 8. AVERAGE AND RANGE OF WATER QUALITY CHEMICAL MEASUREMENTS IN TEST SOLUTIONS OF WATER-EFFECT RATIO ACUTE TOXICITY TESTS CONDUCTED ON AUGUST 30 - SEPTEMBER 1, 2011 USING *Ceriodaphnia dubia*.

PARAMETER	Laboratory Water (DMW)		Site Water (100% effluent)	
	Average	Range	Average	Range
pH, SU	7.7	7.5 - 7.7	7.9	7.7 - 8.0
Dissolved Oxygen, mg/L	8.0	7.4 - 8.6	7.8	6.3 - 8.6
Temperature, °C	24.8	24.6 - 25.0	24.3	24.1 - 24.6
Specific Conductivity, μmhos/cm	177	163 - 184	979	909 - 1043

TABLE 9. RESULTS OF THE 48-HOUR *Ceriodaphnia dubia* ACUTE TOXICITY TEST USING COPPER IN DMW THAT WAS CONDUCTED MAY 12-14, 2011.

Nominal Copper Concentrations, µg/L	Measured Copper Concentrations, µg/L				Mortality No. Dead/Total
	Total Recoverable Day 0	Dissolved			
		Day 0	Day 2	Day 0 and 2 Geometric Mean	
DMW Control	<0.406	<0.406	<0.406	<0.406	0/20
3.4	3.39	2.97	3.32	3.14	1/20
4.8	3.84	4.69	3.99	4.33	0/20
6.9	6.34	6.32[A]	5.70	6.00	12/20
9.8	9.26[A]	9.06	8.81	8.93	16/20
14.0	12.8	13.6	12.5	13.0	20/20
20.0	NA	NA	NA	NA	20/20
LC_{50} →	6.372	6.257[B]			

[A] - Calculated from the geometric mean of two duplicate samples.
[B] - Calculated from the geometric mean of the Day 0 and Day 2 dissolved measurements.
NA – Not available

TABLE 10. RESULTS OF THE 48-HOUR *Ceriodaphnia dubia* ACUTE TOXICITY TEST USING COPPER IN THE SITE WATER (10% EFFLUENT + 90% UPSTREAM WATER) CONDUCTED MAY 12-14, 2011.

Nominal Copper Concentrations, µg/L	Measured Copper Concentrations, µg/L				Mortality No. Dead/Total
	Total Recoverable Day 0	Dissolved			
		Day 0	Day 2	Day 0 and 2 Geometric Mean	
Site Water Control	2.46	2.51	2.26	2.38	0/20
8.0	NA	NA	NA	NA	0/20
11.4	NA	NA	NA	NA	0/20
16.3	17.9	17.1	16.0	16.5	0/20
23.3	24.7	22.8	21.3	22.0	0/20
33.3	35.4	32.8 [A]	29.7	31.2	0/20
47.6	49.6[A]	47.2	42.5	44.8	19/20
68.1	67.4	65.3	60.1	62.6	20/20
97.2	NA	NA	NA	NA	20/20
138.9	NA	NA	NA	NA	20/20
198.4	NA	NA	NA	NA	20/20
LC_{50} →	42.58	38.04[B]			

[A] - Calculated from the geometric mean of two duplicate samples.
[B] - Calculated from the geometric mean of the Day 0 and Day 2 dissolved measurements.
NA – Not available

TABLE 11. RESULTS OF THE 48-HOUR *Ceriodaphnia dubia* ACUTE TOXICITY TEST USING COPPER IN DMW THAT WAS CONDUCTED JULY 7-9, 2011.

Nominal Copper Concentrations, µg/L	Measured Copper Concentrations, µg/L				Mortality No. Dead/Total
	Total Recoverable Day 0	Dissolved			
		Day 0	Day 2	Day 0 and 2 Geometric Mean	
DMW Control	<0.406	<0.406	0.81[C]	<0.406	0/20
3.4	2.90	3.76	3.17	3.45	0/20
4.8	4.34	4.17	3.72	3.94	0/20
6.9	6.13	6.37[A]	6.14	6.25	5/20
9.8	8.60[A]	8.82	11.6[C]	8.82	20/20
14.0	NA	NA	NA	NA	20/20
20.0	NA	NA	NA	NA	20/20
LC_{50} →	6.670	6.710[B]			

[A] - Calculated from the geometric mean of two duplicate samples.
[B] - Calculated from the geometric mean of the Day 0 and Day 2 dissolved measurements.
[C] - Value was considered invalid (analytical error) and disregarded for the LC_{50} calculation.
NA – Not available

TABLE 12. RESULTS OF THE 48-HOUR *Ceriodaphnia dubia* ACUTE TOXICITY TEST USING COPPER IN THE SITE WATER (50% EFFLUENT + 50% UPSTREAM WATER) CONDUCTED JULY 7-9, 2011.

Nominal Copper Concentrations, µg/L	Measured Copper Concentrations, µg/L				Mortality No. Dead/Total
	Total Recoverable Day 0	Dissolved			
		Day 0	Day 2	Day 0 and 2 Geometric Mean	
Site Water Control	15.5	15.2	15.2	15.2	0/20
8.0	NA	NA	NA	NA	0/20
11.4	NA	NA	NA	NA	0/20
16.3	NA	NA	NA	NA	0/20
23.3	40.9	39.3	36.8	38.0	1/20
33.3	48.9	49.7[A]	45.9	47.8	0/20
47.6	64.7[A]	64.6	57.5	60.9	0/20
68.1	87.2	84.2	76.8	80.4	0/20
97.2	114.0	109.0	99.8	104.3	0/20
138.9	157.0	153.0	139.0	145.8	20/20
198.4	NA	NA	NA	NA	20/20
LC_{50} →	133.6	123.1[B]			

[A] - Calculated from the geometric mean of two duplicate samples.
[B] - Calculated from the geometric mean of the Day 0 and Day 2 dissolved measurements.
NA – Not available

TABLE 13. RESULTS OF THE 48-HOUR *Ceriodaphnia dubia* ACUTE TOXICITY TEST USING COPPER IN DMW THAT WAS CONDUCTED AUGUST 30 – SEPTEMBER 1, 2011.

Nominal Copper Concentrations, µg/L	Measured Copper Concentrations, µg/L				Mortality No. Dead/Total
	Total Recoverable Day 0	Dissolved			
		Day 0	Day 2	Day 0 and 2 Geometric Mean	
DMW Control	<0.406	<0.406	<0.406	<0.406	0/20
3.4	2.32	2.28	2.02	2.15	0/20
4.8	2.82	3.10	2.49	2.78	1/20
6.9	4.61	5.07[A]	4.09	4.56	12/20
9.8	7.29[A]	7.15	5.96	6.53	20/20
14.0	NA	NA	NA	NA	20/20
20.0	NA	NA	NA	NA	20/20
LC_{50} →	4.270	4.178[B]			

[A] - Calculated from the geometric mean of two duplicate samples.
[B] - Calculated from the geometric mean of the Day 0 and Day 2 dissolved measurements.
NA – Not available

TABLE 14. RESULTS OF THE 48-HOUR *Ceriodaphnia dubia* ACUTE TOXICITY TEST USING COPPER IN THE SITE WATER (100% EFFLUENT) CONDUCTED AUGUST 30 – SEPTEMBER 1, 2011.

Nominal Copper Concentrations, µg/L	Measured Copper Concentrations, µg/L				Mortality No. Dead/Total
	Total Recoverable Day 0	Dissolved			
		Day 0	Day 2	Day 0 and 2 Geometric Mean	
Site Water Control	36.1	35.4	33.4	34.4	0/20
8.0	NA	NA	NA	NA	0/20
11.4	NA	NA	NA	NA	0/20
16.3	NA	NA	NA	NA	0/20
23.3	NA	NA	NA	NA	0/20
33.3	69.0	66.6[A]	NA	NA	0/20
47.6	84.6[A]	81.0	79.2	80.1	0/20
68.1	104.0	100.0	102.0	101.0	0/20
97.2	132.0	128.0	129.0	128.5	0/20
138.9	179.0	169.0	172.0	170.5	2/20
198.4	212.0	220.0	217.0	218.5	7/20
LC_{50} →	>212.0	>218.5[B]			

[A] - Calculated from the geometric mean of two duplicate samples.
[B] - Calculated from the geometric mean of the Day 0 and Day 2 dissolved measurements.
NA – Not available

TABLE 15. AVERAGE AND RANGE OF WATER QUALITY CHEMICAL MEASUREMENTS IN TEST SOLUTIONS OF WATER-EFFECT RATIO ACUTE TOXICITY TESTS CONDUCTED ON JULY 7-9, 2011 USING FATHEAD MINNOWS.

PARAMETER	Laboratory Water (Moderately Hard Water)		Site Water (50% effluent+50%upstream)	
	Average	Range	Average	Range
pH, SU	7.7	7.5 - 7.8	8.1	7.8 - 8.4
Dissolved Oxygen, mg/L	7.4	6.7 - 7.9	7.5	6.2 - 8.4
Temperature, °C	25.0	24.6 - 25.3	24.9	24.6 - 25.0
Specific Conductivity, µmhos/cm	310	296 - 320	770	741 - 781

TABLE 16. RESULTS OF THE 48-HOUR FATHEAD MINNOWS ACUTE TOXICITY TEST USING COPPER IN MODERATELY HARD WATER THAT WAS CONDUCTED JULY 7-9, 2011.

Nominal Copper Concentrations, µg/L	Measured Copper Concentrations, µg/L				Mortality No. Dead/Total
	Total Recoverable Day 0	Dissolved			
		Day 0	Day 2	Day 0 and 2 Geometric Mean	
Moderately Hard Water Control	<0.406	<0.406	<0.406	<0.406	0/20
63.0	62.3	61.9	55.2	58.5	8/20
90.0	87.3	85.2	78.6	81.8	7/20
128.6	115.0	110.5[A]	99.3	104.7	12/20
183.8	171.5[A]	167.0	148.0	157.2	14/20
262.5	226.0	224.0	210.0	216.9	18/20
375.0	340.0	321.0	273.0	296.0	20/20
LC_{50} →	94.80	88.10[B]			

[A] - Calculated from the geometric mean of two duplicate samples.
[B] - Calculated from the geometric mean of the Day 0 and Day 2 dissolved measurements.
NA – Not available

TABLE 17. RESULTS OF THE 48-HOUR FATHEAD MINNOWS ACUTE TOXICITY TEST USING COPPER IN THE SITE WATER (50% EFFLUENT + 50% UPSTREAM WATER) CONDUCTED JULY 7-9, 2011.

Nominal Copper Concentrations, µg/L	Measured Copper Concentrations, µg/L				Mortality No. Dead/Total
	Total Recoverable Day 0	Dissolved			
		Day 0	Day 2	Day 0 and 2 Geometric Mean	
Site Water Control	15.9	16.4	17.2	16.8	0/20
121.1	NA	NA	NA	NA	0/20
172.9	NA	NA	NA	NA	2/20
247.1	NA	NA	NA	NA	0/20
352.9	357.0	345.0	310.0	327	0/20
504.2	511.0	481.4[A]	430.0	455	1/20
720.3	729.8[A]	697.0	626.0	661	5/20
1029	1080	930.0	813.0	870	3/20
1470	1430	1260	1030	1139	5/20
2100	2130	1330	1120	1220[B]	3/20
3000	3030	1120	1020	1069	4/20
LC_{50} →	>3030	>1220[B]			

[A] - Calculated from the geometric mean of two duplicate samples.
[B] - LC_{50} (>1220) is based upon the highest measured copper concentration since no test concentration had greater than 50 percent mortality.
NA – Not available

TABLE 18. SUMMARY OF THE WERs CALCULATIONS BASED ON DISSOLVED COPPER.

Test Organism	***C.dubia***	***C.dubia***	**FHM**	***C.dubia***
Sample Month/Site Water (%Effl/%up)	**May (90/10)**	**July (50/50)**	**July (50/50)**	**August (100/0)**
Lab Water hardness, mg/L	84.00	84.00	84.00	85.31
Site water hardness, mg/L	269.3	225.3	225.3	169.3
Lab Water dissolved Cu LC_{50}, µg/L	6.257	6.710	88.10	4.178
Lab Water dissolved Cu LC_{50}, µg/L (Hard. Adjusted)	18.75	17.00	223.2	7.969
Site water dissolved Cu LC_{50}, µg/L	38.04	123.1	1220	218.5
Dissolved Cu WER	2.028	7.241	5.466	27.42

TABLE 19. SUMMARY OF THE WERs CALCULATIONS BASED ON TOTAL RECOVERABLE COPPER.

Test Organism	***C.dubia***	***C.dubia***	**FHM**	***C.dubia***
Sample Month/Site Water (%Effl/%up)	**May (90/10)**	**July (50/50)**	**July (50/50)**	**August (100/0)**
Lab Water hardness, mg/L	84.00	84.00	84.00	85.31
Site water hardness, mg/L	269.3	225.3	225.3	169.3
Lab Water total Cu LC_{50}, µg/L	6.372	6.670	94.80	4.270
Lab Water total Cu LC_{50}, µg/L (Hard. Adjusted)	19.10	16.90	240.2	8.145
Site water total Cu LC_{50}, µg/L	42.58	133.6	3030	212.0
Total Cu WER	2.230	7.906	12.62	26.03

TABLE 20. SUMMARY OF THE WERs AND hWERs BASED ON DISSOLVED COPPER.

Test Organism	***C.dubia***	***C.dubia***	**Fathead minnow**	***C.dubia***
Sample Month/ Site Water (%Effl/%up)	**May (10/90)**	**July (50/50)**	**July (50/50)**	**August (100/0)**
CCC (OMZA)	20.50	20.50	20.50	20.50
WER	**2.028**	**7.241**	**5.466**	**27.42**
eFlow	0.1	0.5	0.5	1.0
uFlow	0.9	0.5	0.5	0
uConc	1.26	0.93	0.93	0
HCME	**404.4**	**296.0**	**223.2**	**562.1**
eFlowdf	0.928	0.928	0.928	0.928
uConcdf	1.1	1.1	1.1	1.1
uFlowdf	0.1	0.1	0.1	0.1
hWER	**17.81**	**13.04**	**9.83**	**24.76**

TABLE 21. SUMMARY OF THE WERs AND hWERs BASED ON TOTAL RECOVERABLE COPPER.

Test Organism	***C.dubia***	***C.dubia***	**Fathead minnow**	***C.dubia***
Sample Month/ Site Water (%Effl/%up)	**May (10/90)**	**July (50/50)**	**July (50/50)**	**August (100/0)**
CCC (OMZA)	21.40	21.40	21.40	21.40
WER	**2.230**	**7.906**	**12.62**	**26.03**
eFlow	0.1	0.5	0.5	1.0
uFlow	0.9	0.5	0.5	0.0
uConc	1.51	0.91	0.91	0.00
HCME	**463.6**	**337.5**	**539.2**	**557.0**
eFlowdf	0.928	0.928	0.928	0.928
uConcdf	1.2	1.2	1.2	1.2
uFlowdf	0.1	0.1	0.1	0.1
hWER	**19.56**	**14.24**	**22.75**	**23.50**